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COLCHICINE AND X-RAYS IN THE TREATMENT OF PLANT AND ANIMAL OVERGROWTHS

MICHAEL LEVINE
Montefiore Hospital, New York

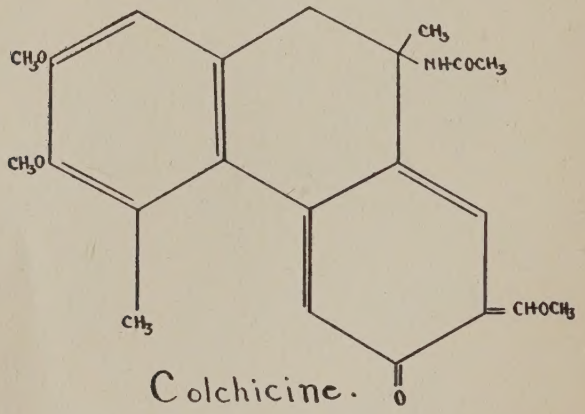
INTRODUCTION

Colchicine, the alkaloid extracted from the seed and corm of *Colchicum autumnale* L., the meadow saffron, has been known for many years. Houdé, in 1887, isolated the substance as a yellow powder or flocculent soluble in cold water, alcohol, and chloroform. The drug found its way into the practice of medicine in the treatment of gout, yet its physiological activities in this disease have not been well defined. When injected into the body in appropriately small doses, it produces gastrointestinal symptoms, such as vomiting and diarrhea. In some respects it behaves like the alkaloid muscarine extracted from the fly mushroom, *Amanita muscaria*. Like pilocarpine, muscarine and physostigmine, colchicine excites nerve endings to glands and smooth muscles. In other respects it behaves like snake poison and toxins; it causes death after narcosis, during which reflexes disappear and respiratory failure follows. Lits and his associates (1938a) give a detailed account of the early uses of this alkaloid. Chemically, colchicine is composed of $C_{22}H_{25}NO_6$. According to Windhaus, the structural formula has a phenanthrene nucleus and is shown on the following page.

Dustin (1933, 1934) studied the action of arsenicals and colchicine on karyokinesis and cytokinesis in tumor tissue. These reports resulted in the development of a number of principal lines of experimental studies. Since Dustin's work, colchicine has been found effective in producing genetic changes in plants. The chromosome number in plants has been polyploidized and mutations have been produced at will. Colchicine has been used as a device to facilitate the study of the effects of hormones on various tissues of the animal body. It has been used as an aid in cytological studies, and has stimulated an interest in a search for other chemicals

which affect the normal processes in nuclear and cell divisions. Finally, this chemical coupled with x-ray irradiation has been used as a therapeutic agent in the treatment of cancer of man and animal, and bacterial overgrowths on plants.

These different phases of colchicine literature are reviewed separately and referred to below. The large number of reports now extant make it impractical to include all of them in a general survey. Here, only the salient features will be mentioned, and emphasis given to those studies which deal with the effects of this alkaloid alone or in combination with x-rays on animal and plant hyperplasias.



Colchicine.

The early studies on the effects of colchicine on cancer were fragmentary and without experimental basis. The belief that colchicine was an ameliorative in cancer seems to have been derived from some observations on gout patients who were concurrently suffering from cancer. In "Peau, Syphilis, Cancer" (1932) mention was made of Dominici's observations of cancer patients suffering from gout. He claimed these patients showed improvement and that the cancerous condition was arrested after treatment with colchicine. Dixon and Malden (1908) were the first to study the pharmacological properties of colchicine on the blood and bone marrow of rabbits, rats, dogs and on one or two cases of man. Rabbits injected subcutaneously with 1 cc. of 0.5% solution of colchicine caused transient leucopenia and a diminution in the polymorpho-

nucleate cell counts. Administration of large doses induced abnormal cells, and normoblasts were then commonly found. Small doses when repeated over several months produced no ill effects; the leucocyte counts remained unchanged. Treatments extending over long periods, however, produced definite changes in the bone marrow. The marrow cells increased and became more conspicuous, and fat cells disappeared. Very large doses of colchicine, the authors contended, produced destruction of all blood elements. Bone marrow elements were observed in the general circulation. Marrow smears showed an abundance of mitotic cell divisions. The significance of these observations was not apparent to Dixon and Malden.

The study of the mechanism of nuclear and cell division and attempts to alter their course have been in progress many years. Dustin had long been studying the action of various chemicals on cell division, and in 1933, in collaboration with Grégoire, he investigated the effects of sodium cacodylate and trypaflavine on mitosis of the well known mouse sarcoma, 180. Dustin (1934) continued these studies, using trypaflavine, rivanol, isamine blue, malachite green, arsenicals, benzol, iodine, zinc, mercury, thallium and certain microbic toxins which he described as "caryoclasiques" agents. Sodium cacodylate and typaflavine effects were studied on tumor grafts of the Crocker mouse sarcoma. The method employed by Dustin was subsequently used with modification by many investigators. It consisted of injecting small quantities of the chemical (30 mg. per 20 gm. of body weight) into the tumor grafts when they had attained the size of a cherry. The animals were sacrificed at intervals of 3 to 4 hours for the first day and at 48 hour intervals afterwards. Counts of the number of mitoses in the mouse sarcoma after sodium cacodylate injections, reached a maximum at 24 hours but showed a reduction at the 48th hour. Dustin (1934) believed that 0.025 mg. of colchicine had a stimulative effect on cell division in the sarcoma grafts much like that produced by sodium cacodylate. The number of nuclear divisions was calculated to be fifteen times greater than in the untreated tumor tissue. These divisions were followed by karyorrhexis. Colchicine, Dustin believed, produced the maximum number of division stages in the Crocker tumor at the ninth hour after injection. Similar reactions were observed in the Kupffer cells, reticulo-endothelial cells, the megocytes and testicular cells.

Dustin (1937) studied, further, the effects of numerous chemicals on cell divisions and believed the agents examined could be arranged into two classes, those that acted like trypaflavine and those that acted like arsenical compounds. The first class he characterized as one having essentially an inhibiting effect on karyokinetic division and resulting in "caryoclasic" shock. Substances in the cacodylate group, he found, produced reactions which he considered as violent excitants of mitotic division. He described the cytoplasm of these cells as turgescent, the prophase stages as of short duration followed by atypical metaphase stages. The prolonged metaphase stage, which became diagnostic for colchicine treatments, showed chromosomes markedly condensed, forming compact masses, which he described as "radiomimetic." These mitoses either disintegrated or completed the division of chromosomes without aid of a spindle structure. This division was frequently followed by development of "monstrous" nuclei. Dustin believed that malignant cells were highly sensitive to this alkaloid. Dustin (1938), too, showed that the dosage of colchicine required to bring about extreme mitotic activity in tissue of the mouse was a very small fraction of that required for the cacodylate. He found that reaction occurred principally in the generative zone or in cells at division induced by various conditions, such as trauma, inflammation, carcinogenic activity or hormones. Achromatic figures, asters and spindles were totally lacking in these colchicinized cells. While some cells showed complete pycnosis, normal telophase stages and cytoses occurred. Some cells assumed normal telophase stages and formed giant or polyploid nuclei. Dustin proposed the name of "stathmocinesis" for this type of indirect division and applied the name of "stathmocinetic poison" to colchicine. He contended that the arrest of the nuclear division in the metaphase was preceded by a phase of excitation which distinguished this poison from those which merely inhibited division.

Delcourt (1939a, b), working in Dustin's laboratory, studied the effects of colchicine (0.5 mg. to 250 mg.) at ordinary temperature on tissues of the lower vertebrates, frog, salamander and axolotl. He found that a pycnotic stage precedes excitation of mitosis. The dividing cells acted upon by colchicine showed a complete absence of spindle figures and the chromosomes were clumped as described by Dustin for tumor tissues. Delcourt found that the effects of

feeble doses of colchicine were augmented by higher temperature (37° C.). The maximum response in *Rana* to 0.5 mg. of colchicine occurred at 162 hours, while axolotl showed the maximum number of division stages at 183 hours after 0.025 mg. of colchicine.

COLCHICINE ON PLANTS

Havas (1937a), another of Dustin's collaborators, studied colchicine effects on plants inoculated with the plant tumor-producing organism, *Bacterium tumefaciens*. The stems of tomato plants inoculated with the bacteria were cut so as to free a spur of tissue by partial cross section near the base with longitudinal cuts extending for short distances up the stem. The spur on each plant was inserted on alternate days into a 1 : 10,000 solution of colchicine. Distilled water was used on the other days. Havas observed that colchicine stimulated the appearance of tumors, but when the end results were examined he found that the tomato stems treated with colchicine produced tumors that were but half as heavy as those of the controls. Havas' results led him to believe that colchicine was detrimental to the general development of the plant. Tomato crown galls painted with a mixture of lanolin and colchicine, in which the colchicine was 40 mg. per gram of the mixture, produced a reduction in the weight of the tumors as compared with those of the controls. Treatment by the spur immersion method of *Pelargonium* stems bearing tumors had no effect on the size of the tumor. Covering a scarred area of about 1 cm. on begonia stem with 3 mg. of a mixture of lanolin and colchicine stimulated development of the area, while similar treatment on *Impatiens* proved toxic in some cases though resistant specimens produced adventitious roots. Havas believed that colchicine acted as a local excitant of growth similar in action to that of a hormone. Colchicine, he contended, inhibited the development of plant tumors or crown gall on tomato. Havas (1937b) studied, further, the effects of colchicine on germinating seeds and seedlings of a pure line of Wilhelminia wheat. Roots and root hair development were stimulated at first, but the effects were followed by a depression in the growth rate and finally complete arrest. Colchicine effects were shown in these seedlings by the development of bulbous hypertrophy of the root tips and liquefaction of certain parenchymatous elements. Havas (1938) later attempted to show that colchicine effects were of a hormonal

nature, as evidenced by the appearance of adventitious roots, "pseudo-neoplasms" on root tips, hypertrophy of the coleoptile in seedlings, and stimulation of somatic growth of *Begonia*. Tomato plants showed epinasty when the leaves were stroked with the finger dipped in a 5:1,000 solution of colchicine. The response developed in 40 minutes and lasted four to six hours. *Tradescantia* showed a hyponastic reaction when sprayed with a 1:20,000 solution of the drug. Wounds on cherry laurel leaves healed in a few hours when treated with 1:2,000 to 2:1,000 colchicine solution. Havas believed he was dealing with a "phytohormone" comparable with the wound hormone, traumatin. Yeast was stimulated to growth by colchicine and thus behaved like bios or vitamin B, according to Havas. Yet he concluded that colchicine was not a hormone but a mobilizer of the hormone already in the plants. The hypertrophies produced on root tips Havas (1939) believed analogous to the crown gall disease.

The contention of Dustin and his associates that colchicine has a therapeutic value in tumor growth resulted in many studies designed especially to determine this point. Animal pathologists directed their attention to the effects of colchicine on a number of different types of neoplasia in animal and man. The plant disease known as crown gall first tested with colchicine by Havas was also studied intensively in this connection. So far, no other type of plant overgrowth commonly referred to by botanists as plant tumors, such as the potato wart, club root and nematode galls, has been investigated in its relation to colchicine. Brown (1939), who was one of the first investigators associated with experimental production of crown gall, made a series of investigations to determine the therapeutic effects of colchicine on these bacterial overgrowths. She attempted to prevent crown gall formation as well as to destroy it when fully formed. Eight species of plants were under observation, Paris daisy, French marigold, four o'clock, *Bryophyllum, Kalanchoë, Nicotiana glauca,* kidney bean and tomato. Plants treated with colchicine by the Havas method failed to prevent formation of crown gall. A delay in tumor formation was observed when the concentration of the colchicine solution used was 0.5% to 2%. In some cases the tumors formed were smaller than in the controls; in other trials the colchicine injured the plants severely. Those plants which were inoculated with the bacteria and then injected with

0.2 cc. to 0.5 cc. of a 2% solution of colchicine produced nine tumors in 61 individuals of five species of plants. Colchicine dwarfed tomato and marigold plants, although these effects appeared to be temporary in the tomato. The areas inoculated with *B. tumefaciens* were covered with lanolin to which 3% colchicine was added. Brown found that this treatment delayed tumor formation but that the tumors grew to more than half the size of the controls. Overgrowths produced by indoleacetic acid when similarly treated with colchicine produced like results. Brown concluded that colchicine did not prevent the formation of overgrowths after chemical or bacterial treatment.

In an attempt to kill or prevent further development of fully grown crown galls, a colchicine solution was painted on the surface of these growths or a lanolin colchicine paste was applied. Growth of crown gall on Paris daisy was inhibited and the tissue blackened in 16 days; at the end of 70 days the galls were dead, although the stems above and below the overgrowths were alive. A more interesting observation made by Brown concerned the failure of 0.5% solution of colchicine to cause death of tumors on the tomato plant, although a 2% solution interfered with the growth of the galls so that controls were twice the size of the treated ones. *Kalanchoë* stems bearing crown galls set in 0.5% solution of colchicine for four hours and then transferred to water for ten days produced roots at the galls, while controls showed no roots; the treated tumors blackened and died.

Brown believed that death of the galls was not due to direct killing or inhibiting actions of colchicine on the bacterial organisms, but, in accord with Havas, contended that the colchicine affected the growth substances in the plant.

Brown (1942) further studied the effects of acenaphthene, methylnaphthalene, α-nitronaphthalene, 3,5-dibromopyridine, heptyl aldehyde and apiole. These substances were incorporated in a paste made of lanolin. Apiole induced dwarfing of the treated plant but no blackening of the tissue nor death; α-methylnaphthalene (50% and 100%) inhibited growth; heptyl aldehyde, as well as α-methysalicylate, used in full strength caused death. The age of the tumor, Brown pointed out, affected the survival rate of the tumors; young tumors were inhibited, old tumors were killed. Dermen and Brown (1940a, b) studied the cytological effects of

colchicine on plant tumors. Twenty day-old tumors on African marigold and French marigold were used, to which various solutions of colchicine varying from 0.025% to 1% were painted on the surface of the gall. The treated tissues were removed at intervals of 2, 5, 7 and 9 days after treatment. The cellular changes found were associated with excessive polyploidy and multiploidy in the affected meristematic cells of the tumor tissue. The effects induced by the colchicine were not immediate for growth in controls, and treated tumors continued for a week. Then the treated tumors blackened and eventually died. The principal cytological change which accompanied these morphological ones was multiploidy. This condition resulted in large nuclei in hypertrophied cells of the treated tissue. The size of the cells so produced had definite limits, the authors believed, beyond which death followed. Dermen and Brown suggested repeated colchicine injections in conjunction with irradiation as a therapeutic measure for cancer.

Solacolú, Constantinesco and Constantinesco (1939) treated crown galls on stems of *Pelargonium zonale* and *Ricinus communis* with colchicine. The galls were covered with a paste of lanolin hydrate in which 0.5% to 0.75% colchicine was introduced. This treatment was repeated three or four times at 15-day intervals, and as a control lanolin was used alone. These authors observed a layer of meristem tissue formed between the tumor and the host. This new tissue became suberized and thus cut the metabolic exchange between the plant and the tumor so as to kill the new growth.

COLCHICINE ON ANIMALS

Lits (1936), another of Dustin's associates, investigated the cellular lesions in animals produced by this alkaloid. Injection of rat tumor with the chemical frequently resulted in the production of cells too abnormal to be viable. Aberrant cells frequently met in malignant growth were destroyed. Lits believed that the cytological effects of the colchicine resembled those induced by radium. He contended that colchicine was more "caryoclasique" than "caryocinetogenic". Rat tumors, benign or malignant, were found sensitive to the radiomimetic-inducing properties of colchicine. Lits, Kirschbaum and Strong (1938b) emphasized the radiomimetic properties of colchicine attributed to it by Dustin, and studied its effect on lymphoid tumors of the mouse. Mice of the Strong C_3H

strain were injected with leukemic lymphoid tissue, and when the transplants had attained the size of a kidney bean (14 days), 1/40 mg. of colchicine in distilled water was injected subcutaneously. This injection was repeated at intervals of three days. Complete disappearance of the local growth occurred. Survival time of controls after the transplantation was 31.5 days as compared to 50.5 days in colchicine-treated tumors. In no case was there complete regression without recurrence. Histological examination of the tissue after the third injection showed all lymphoid cells of the tumor in pycnotic condition or dead; the reticular cells of the lymphosarcomatous growth remained. Colchicine was found to hasten death in those animals where the lymphoid neoplastic disease was systematic.

Amoroso (1935), observing a beneficial effect of colchicine on gout patients who had been treated for cancer with x-rays, tested the efficacy of colchicine on cancerous growths (Carcinoma M63) on white mice. The tumors on these mice grew slower than the controls, and at the end of two weeks there was no microscopic evidence of them in two thirds of the tumor animals. The remaining third showed complete regression eight weeks later. In another series of similar mouse tumor experiments there was no recognizable tumor development in two weeks after colchicine injections were made. In a spontaneous tumor in the peritonsillar region of a dog, Amoroso recorded complete regression after injection with colchicine. The combined effects of x-rays and colchicine were not studied. Poulsson (1935) used 15 mice with malignant tumors, five spontaneous mammary carcinomas and ten with tar cancer to determine the curative effects of colchicine. He injected 1/80 mg. of colchicine subtucaneously and found that the drug had no effect on development of the tumors. The tumors ran their usual course terminating the life of the animal by ulceration of the tumor with infection and hemorrhages.

Peyron, Lafay and Kobozieff (1936) tested the action of colchicine on the Shope rabbit papilloma. The rabbits were injected eight or nine times over a period of three to four weeks with an average dose of 1.5 mg. of colchicine. The authors reported complete regression of the tumors without recurrence. In a later paper (1937a), in collaboration with Poumeau-Delille, Peyron and Lafay injected rabbits with Shope papilloma with slightly smaller doses

(1 mg.) of colchicine. The rabbits carried tumors of different ages varying from one to four months. The control rabbits, with the exception of the cases of spontaneous regression, went through the usual changes characteristic of this disease followed by death. The colchicine-treated animals were free from the tumors within three to five weeks. In two series of experiments, colchicine was applied in aqueous solution of 1/100 or in paste in concentrations of 1/20, 1/50 and 1/100. In another series the papilloma was introduced into both flanks of the animals. Only the tumor treated with colchicine showed rapid and uniform regression. The untreated tumors became stationary and regression occurred at a much slower rate. Of 19 animals, eight died before the regression of the treated side was completed. These deaths were associated with secondary infection, intoxication and ulceration. In the 11 survivors, regression of the treated tumors was completed or nearly so; six to eight weeks sufficed if the treatment with colchicine was continued. The authors expressed the opinion that colchicine given orally or by local applications might be beneficial in precancerous lesions or benign tumors in man.

Clearkin (1937) studied the effects of subcutaneous injections of colchicine on transplants of mouse sarcoma 37. The animals inoculated with tumor fragments were injected with 0.0015 to 0.003 mg. of colchicine per 20 gm. of body weight. This was followed on alternate days by injection of 0.01 mg. of the alkaloid. No significant difference was noted between these animals and the tumor controls. Mice bearing tumors approximately 1 cm. in diameter were injected with 0.01 mg. of colchicine every other day. In this series no difference was observed between the tumor-bearing animals treated and those not treated. The rate of growth was the same in both groups. Mice were inoculated with bits of tumor from a mouse which had received 0.01 mg. of colchicine; three days later they were injected intravenously with 0.04 mg. of colchicine. One hour after the injection, the transplanted tumor tissue was removed and implanted into fresh hosts. Controls were similarly treated except for omission of the colchicine injection. The tumor tissues from both sets of animals were also cultured *in vitro*. There was no difference in the number of takes of the experimental and control series *in vivo*. There was, however, a slight retardation in the rate of growth of the tumors derived from the colchicine-treated

animals during the first week after their appearance. The end results, however, were similar. Cultures *in vitro,* however, showed that 60% of the colchicinized tumor transplants failed to grow. In the remaining cultures growth was very slight. The controls, *in vitro,* showed vigorous growth in 90% of the cultures. Clearkin believed the sarcoma 37 was less affected by colchicine than many normal tissues.

Ludford (1936) was among the first to study the effects of colchicine on animal tissues. He studied the effects of this and of other chemicals, such as auramine, urethane, methylsulphonal and sodium cacodylate, on mouse carcinoma 63 grown *in vivo* and *in vitro*. Tissue cultures of this tumor grown in a medium to which colchicine in varying concentrations of 1 : 100,000 to 1 : 100 million were investigated. Solution 1 : 100,000 to 1 : 800 inhibited outgrowth of the transplant and arrested cell division. In those cultures in which mitoses were brought to a standstill, the nuclei of the resting cells were appreciably altered. Concentrations of colchicine which inhibited mitoses also affected resting nuclei of normal and malignant tissue. Ludford's experiments showed that the reactions of cells to colchicine or sodium cacodylate were essentially alike but that the former induced reaction at a much lower concentration than that required by the latter. Colchicine, Ludford showed, was active over a wider range of concentrations. Ludford is credited with having pointed out the inhibiting action of colchicine; the stimulative effect at first claimed by Dustin and his coworkers were shown to be an accumulation of the arrested cells in metaphase stage. Schairer (1940) used ascitic cancer of the mouse *in vivo* as the test material. Besides observing the typical colchicine phenomena Schairer noted that the mitotic changes appeared in two hours and the accumulated mitotic figures became prominent at 15 hours. Like Ludford (1936) this author concluded that a curative effect with colchicine could not be attained without injury to the animal.

Tennant and Liebow (1940) also resorted to tissue culture for testing the effects of colchicine and ethylcarbylamine. They believed that by this method the concentration of the chemicals actually in contact with the cells was known. The tissue used consisted of mammary cancer in mice induced by theelin and breast tissue of new-born mice. Colchicine, they found, reduced the ex-

pansion rate of the colonies of the mouse breast cancer in concentration as low as 1 : 64 millions. The rate of mitosis was reduced but the cells in mitosis accumulated. Many cells remained alive, as demonstrated by successful transplants. Ethylcarbylamine acted like colchicine but its activity was confined to a narrower but higher range of concentrations. Bucher (1939) studied the growth rhythms of fibroblasts in tissue cultures. He added trypaflavine or colchicine to cultures of these cells and compared their reactions. Colchicine, Bucher held, retarded the division rhythm with initial injury in two to three hours. Trypaflavine blocked more or less completely the beginning of new mitoses. Bucher believed that the disturbance produced by colchicine or trypaflavine is one of a regulative mechanism.

Gavrilov and von Bistram (1939) studied heart and iris tissue of the chick *in vitro*. Colchicine in low concentrations acted as a specific mitotic poison producing vacuolization and granulation of the cytoplasm. A new form of cell induced by colchicine was described as "monocytoid" which was characterized by a compact and deeply stained nucleus. These cells were derived from macrophages and fibroblasts. When the action of colchicine was not too severe, the "monocytoid" cells recovered and regenerated into macrophages and fibroblasts.

Brues (1936) investigated the effects of colchicine on the regenerating liver of the rat following partial hepatectomy, as a prelude to his studies of the therapeutic value of this drug on cancer. The animals were injected with 0.1 mg. or 0.2 mg. of colchicine per 100 gm. of body weight 22 hours after operation, and then sacrificed at eight hour intervals. Microscopic examination of the livers failed to show a stimulating effect of the alkaloid, as contended by Dustin, although large doses induced abnormal mitoses. Brues and Cohen (1936) studied the effects of colchicine and colchicine derivatives on cell division. They found that a comparable dose of sodium cacodylate gave essentially the same cellular responses as colchicine. Partially hepatectomized rats were injected with an aqueous or oil solution of colchicine varying in strength from 0.1 mg. to 1 mg. per 100 gm. of body weight of the rat. With the smallest doses the number of arrested mitoses was small, and while most of the chromosomes were clumped, some cells showed scattering of chromosomes. With injections of 0.2 mg. of colchi-

cine, optimum results were obtained for the number of arrested mitoses. The largest dose (1 mg.) caused death coupled with the smallest number of mitoses. All the colchicine derivatives used (octahydro-colchicine, N-acetylocolchicine and 4 derivatives of the latter compound) produced effects similar to those of colchicine, but only when applied in considerably higher doses. Dimethyl and trimethyl colchicine acid were found effective in arresting mitoses in any sublethal or lethal dose. Scheifley and Higgins (1940) also studied the effects of partial hepatectomy on male white rats in which 70% of the liver was removed. The toxic effect of the colchicine as measured by mortality was found to be inversely proportional to the amount of the liver present.

COLCHICINE AND X-RAYS

Brues and Jackson (1937) studied the nuclear abnormalities resulting from inhibition of mitosis induced by colchicine and other substances. Rat sarcoma was studied ten hours after an injection of 0.025 mg. of colchicine. The peripheral cells in the tumor showed the greatest number of mitotic figures. Brues and Jackson believed the size of the dose determined the degree of aberrant cytological behavior. In a later paper, Brues, Marble and Jackson (1940) studied the effects of daily treatments of colchicine on animal tumors and regenerating tissue. They believed that a narrow range of dosage existed which caused the characteristic effect upon cell division and that this dosage could be injected daily without causing death of the animal. The belief that cells in mitosis were most susceptible to the effects of Roentgen radiation led them to study the influence of x-rays on colchicinized tissue. Radical operations were performed in an effort to affect recurrences and metastases from small nests of cells which the alkaloid might readily penetrate. Four groups of experiments were arranged; a control, a group in which only colchicine was injected, a third group in which the tumor was irradiated and a group in which the combined treatments of colchicine and x-irradiations were administered. The exposure to x-rays were made in ten daily treatments usually of 300r each. The x-rays were given 16 hours after the colchicine was injected. The results obtained in the colchicine series alone showed no regression but the tumors remained quiescent or retarded. No satisfactory statistical analysis of growth rate differences between this

group and the controls could be made. In the study of the combined effects of colchicine and irradiation the results were different in two mouse tumors, C67 and 180. Tumor 180 showed only a slight regression after 3000r. The mouse tumor ceased to grow four to five days after beginning of irradiation and assumed its usual growth three to ten days after the irradiation was discontinued. In the Walker rat tumor, five tumors of 24 regressed permanently and one disappeared temporarily. Rat tumors ceased to grow on the second to fifth day after beginning of irradiation and remained quiescent for seven to ten days, after which they disappeared rapidly or resumed growth slowly. There was little difference between the tissues irradiated only and the tissues given the combined colchicine and irradiation treatment. Brues and his associates believed that colchicine had no effect on the response of these tumors to irradiation. Regression began no sooner after irradiation where colchicine was given than where it was not given. Colchicine did not even decrease the latent period between irradiation and various stages of regression as might be expected of regression dependent upon destruction of cells which were in mitoses at the time the irradiation was given.

Guyer and Claus (1939) used 672 rats with Flexner-Jobling carcinoma in a series of experiments to test the combined effects of colchicine and x-rays on this tissue. They found that 0.1 mg. of colchicine per 100 gm. of body weight administered subcutaneously coupled with irradiation of 3,000r proved a lethal dose to these animals in two to three weeks; 4,500r given in three doses of 1,500r each administered at intervals of two weeks or more were better tolerated, but smaller doses of 188r to 375r, frequently repeated, proved more effective than the larger doses. They counted the number of divisions in the tissue at various periods after the injection of colchicine. They found that rat tumor tissue prior to colchicine injection showed an average of 6.3% mitotic figures in 3,000 cells counted, while 15 hours after the colchicine injection the mitoses increased to 38.2%, which represented the highest number obtained. In another experiment the tissues from colchicinized tumor-bearing rats were removed, cut into equally thin slices and exposed simultaneously to 1,500r. This tissue was then transplanted into normal rats. All of the 89 rats classified as nontakes were later implanted with non-treated tumor grafts; 63 of

these animals developed tumors. Guyer and Claus believed they showed that far greater lethal effect was produced by the combined effects of colchicine and x-rays on cancer tissue than by x-ray treatment alone or of colchicine alone. In studying the combined effects of colchicine and x-rays on living rats bearing tumors, 91 animals were used. These were divided into three groups; 32 were treated with colchicine and x-rays, 29 with x-rays, and 30 with colchicine. The x-rays were given in small doses of 188r twice weekly. The x-ray treatments were started 16 days after the implanted tumor attained the size of 1 cm. to 1.5 cm. and 15 hours after the colchicine was injected. At the end of six weeks, the group which received both x-rays and colchicine showed a large number of animals healed and few deaths were recorded. Guyer and Claus concluded that x-rays were markedly more lethal to bits of cancer tissue from colchicine-treated animals than to comparable pieces of cancer of untreated animals, as shown by infrequency of takes on transplantation to susceptible animals. Administering colchicine to rats bearing actively growing tumors 15 to 18 hours before irradiation, x-rays were found effective in completely destroying or in retarding the tumor growth.

In a later paper, Guyer and Claus (1940) studied the effects of injected distilled water on Flexner-Jobling rat carcinoma 15 hours after subcutaneous injection of colchicine. The water was injected into the tumors. After one week the surface of the tumors sloughed off and many of the growths appeared to be healing. Repeated injections of distilled water were made at intervals of two weeks. In four series of experiments 103 rats were used and healing was observed in 59 cases (57.3%); 18 tumors remained unhealed; 26 animals died.

Oughterson, Tennant and Hirschfeld (1937) reported the effects of subcutaneous or intramuscular injections of colchicine on 21 cancer patients. Of this number, 15 permitted biopsies before the alkaloid was administered. After the colchicine was injected biopsy material was again taken or the entire tumor was removed nine and a half hours after the injection. The doses of colchicine varied from 1 mg. to 4 mg., and as little as $1\frac{1}{2}$ mg. produced the cellular changes typical of colchicine effects. In some cases the authors were able to secure frequent biopsies which enabled them to count 19.6 cells in metaphase stage per high power field 12 hours

after the administration of colchicine as compared to 2.6 mitoses in the controls. Oughterson, Tennant and Laurence (1940) studied the effects of x-rays on a transplantable mammary carcinoma in strain A mice. Irradiating with 5,000r, cures were effected in 48% of the mice, while hybrid mice showed cures with 2,500r and 5,000r, respectively, varying from 82% to 97%. Tumors that responded best to x-ray therapy, they believed, developed a characteristic stroma. They considered the host an important factor in determining the response to irradiation. In a later paper, Hirschfeld, Tennant and Oughterson (1940) studied the combined influence of colchicine and x-rays on a strain of mice with transplantable mammary carcinoma and a breast tumor of a male mouse resulting from injections of oestrone. The mice were treated when the tumor transplants attained 0.5 cm. to 0.7 cm. The colchicine dose was calculated in milligrams per gram of body weight and administered in 5 cc. of saline. The effects of various doses of the alkaloid were studied. Attempts to cause regression of the tumors with single or repeated doses of the drug were also made. The colchicine had no effect on regression of the spontaneous tumors of strain A mice. The combined effects of colchicine and x-rays on the growth of the transplantable mammary tumors were studied. The authors contended that a single dose of 0.0008 mg. of colchicine per gram of body weight produced contracted metaphases which appeared most abundant at four, five and six hours after injection. When the animals were injected with 0.0007 mg. per gram of body weight and this dose was repeated nine times at intervals of nine hours, a marked increase in mitotic figures was observed at the 27- and 36-hour intervals. Necrosis appeared at 36 hours; at 81 hours all the animals died. Injection of 0.0016 mg. of colchicine at three- to five-day intervals caused retardation of growth and prolongation of life of the tumor animals. Animals with mammary tumors 0.4 cm. to 0.5 cm. in diameter were irradiated eight to ten hours after each was given a single subcutaneous injection of 0.0016 mg. or 0.001 mg. of colchicine per gram of body weight. Mice were irradiated eight to ten hours after a second dose of colchicine was administered at an interval of nine and a half hours. In these experiments a dose of colchicine which produced a maximum accumulation of mitoses plus a single exposure to irradiation with 2,500r gave a slightly higher ratio of curability than x-rays alone. How-

ever, these authors believed the results insufficiently significant to warrant further study. Colchicine combined with 5,000r gave results similar to those obtained with x-rays alone. Colchicine, these authors concluded, did not strikingly increase the destructive effects of x-rays upon the tumor.

Seed, Slaughter and Limarzi (1940) studied the effects of colchicine combined with x-rays on advanced carcinomas of man. The four cases reported consisted of two breast adenocarcinomas, one rectal tumor, and one unclassified mass in the neck of a male. The colchicine dose given was repeated at intervals of seven days following x-ray irradiation. Two of the subjects died of colchicine poisoning. Two patients were irradiated, one of whom was treated before the colchicine was administered. Biopsies made after 10 mg. of colchicine were administered showed numerous mitotic figures in metaphase. The tumors necrotized, but after considerable sloughing, growth proceeded rapidly and death followed.

COLCHICINE AND BACTERIAL FILTRATES

Shwartzman (1936a, b) described the production of hemorrhage and necrosis of tumors by injection of bacterial filtrates into animals. Andervont (1940) studied the effects of colchicine and of bacterial products on animal tumors. He also tested the effects of repeated injections of colchicine on the growth of spontaneous mammary tissues and the combined effects of colchicine and bacterial products. Colchicine in large doses (0.15 mg. to 0.2 mg.) caused hemorrhage but also proved lethal, while smaller doses caused correspondingly less hemorrhage without much change in the tumor. Filtrates of *B. coli* passed through an N Berkefeld filter showed wide range of hemorrhage potency. Andervont concluded that colchicine and bacterial filtrates supplemented each other in producing hemorrhage and regression of transplantable mouse tumors, but the combined effects were no greater than that of a single injection of colchicine. Ascorbic acid which prevents tumor hemorrhage after bacterial filtrate injections proved ineffective after colchicine.

Boyland and Boyland (1937–1940) showed that tumor-bearing mice and rats injected with a near lethal dose of colchicine produced hemorrhage in the tumor with a reduction in the ascorbic acid content of the tumor, also a reduction in metabolism. They believed

that filtrates of *B. typhosus* and colchicine resembled each other in the effects they produced. They found that a given dose of colchicine was more toxic to tumor-bearing mice than to normal mice and that ascorbic acid had no favorable effect on the survival rate of tumor mice. Studies were made on transplanted, induced and spontaneous mouse tumors after intraperitoneal injections of colchicine followed by x-rays 18 to 20 hours afterward. Respiration and anaerobic glycolysis were measured and the vitamine C content was determined. The degree of hemorrhage was estimated macroscopically. The authors concluded that the spontaneous tumors appeared much less affected than the grafted tumors and somewhat less than the methylcholanthrene-induced tumors. Boyland and Boyland were the first to observe that ascorbic acid did not neutralize the hemorrhage-producing power of colchicine. They observed that 2-methylnaphthoquinone did not alter the effects produced by colchicine.

ACENAPHTHENE AND OTHER CHEMICALS

The effects of trypaflavine, an arsenical compound, and of colchicine on mitoses in pathological and normal animal and plant tissues stimulated interest in search of other chemicals which would induce similar changes. Dustin, Lits, Brues, Brown and others have studied a number of chemicals with this point in view. Schmuk (1938) and later in association with Gusseva (1939) showed that acenaphthene, like colchicine, could induce cells with multiples of the species-number of chromosomes. The assumption that a certain relationship existed between these substances and the carcinogenes led Schmuk and Gusseva to test many animal tumor-producing substances for their ability to induce polyploidy. The chemicals were dissolved in ether and poured over the filter paper. When the ether evaporated, the paper was moistened with water, and seeds of corn, wheat and barley were placed on it to germinate. Schmuk found that wheat and barley seeds were polyploidized by acenaphthene, acenaphthylene, chloracenaphthene, bromacenaphthene, α-chlornaphthalin, α-bromnaphthalin, α-iodonaphthalin, α-dibromobenzol, methylnaphthalaether, 3,5-dibrompyridin, ethylnaphthoat and α-nitronaphthalin. Germinating legume seeds did not become polyploid from but were resistant to the effects of acenaphthene. Kostoff (1938a) followed Schmuk with studies on

germinating seeds of wheat, rye, oats, barley, maize, rice, *Festuca* and *Lolium*. The seeds were covered with crystals of this compound for four to eight days and allowed to grow slowly. Legumes (*Vicia, Erodium, Lothyrus, Medicago*) reacted slowly after an exposure of six to twelve days. Compositae responded in four to eight days. Kostoff showed that mitoses proceeded abnormally, no spindle fibers were produced, no equatorial plate stages appeared; the chromosomes divided but did not move to the poles, thus forming polyploid nuclei. The chromosomes were spread in the cytoplasm, frequently giving rise to polynucleate cells. Floral buds of nine species of *Nicotiana* were treated with crystals of acenaphthene. Abnormal microspores and abortive pollen grains were found. The viable pollen grains had unequal numbers of chromosomes. Kostoff (1938b) induced chromosome doubling in species of *Triticum* and *Secale* by subjecting the seeds to a saturated solution of acenaphthene with excess of crystals. Acenaphthene, Kostoff stated, acted like colchicine by creating conditions for polyploidy in *Triticum, Nicotiana* and *Lactuca*. He pointed out that long exposures to large amounts of sublimated particles of acenaphthene may injure or even kill plant tissues. Nebel's (1938) results with saturated solutions of acenaphthene on *Tradescantia* gave no polyploidy. Acenaphthene, it must be remembered, is only very slightly soluble in water. The sublimated crystals, it seemed, alone produced reactions. Kostoff (1938c) later concluded that colchicine was toxic, while acenaphthene did not appear to be. In a later report, Kostoff (1939d) induced polyploidy in some series of cereals by subjecting them to pulp from disintegrating corms of *Colchicum autumnale* L., which suggested the possibility of finding polyploid plants among the species growing in nature in association with *Colchicum*. Bates (1939) tested this theory and found that other plants growing among corms of the meadow saffron revealed no abnormalities. In nature, Bates believed the polyploid individuals were suppressed in competition with the normal seedlings or established vegetables.

Gavaudan, Gavaudan and Durand (1938) tested acenaphthene on germinating wheat seedlings. They found themselves in accord with Kostoff in that acenaphthene prevented spindle formation. These investigators also tested the efficacy of diphenyle and naphthalene and their derivatives on dividing cells. While arrested mitoses were observed, some of the derivatives like diphenylmethene

were toxic. Gavaudan (1941) tested (1,2,3,4,5-tetramethylbenzene) durene and found that it induced mitotic inhibition similar to acenaphthene. Gavaudan and Gavaudan (1939b) discovered that apiole, an extract of parsley, inhibited cell division. They found that *Hordeum* seedlings were very susceptible to apiole and showed after the first 24 hours tetraploid and octoploid cells. Karyokinetic anomalies were varied in the species studied. After colchicine, the authors believed, apiole was the second known substance existing in a natural state capable of modifying nuclear and cell division and eventually producing polyploidy.

Simonet and Guinochet (1939a) reported the production of polyploidy in flax seedlings with paradichlorobenzene. This substance, they believe, produced morphological changes similar to those effected by colchicine and acenaphthene. Simonet and Igolen (1940) investigated the effects of the vapor of the oil of leaves of *Citrus nobilis* on flax and on barley seedlings. Roots of the latter were more sensitive than flax. Marked disturbance of cell and nuclear divisions were observed. Anaphase and telophase stages were lacking and the cells became multinucleate. The principal constituent of the oil, methylester of methylanthranilic acid, produced the same reactions. Simonet and Guinochet (1939b) obtained effects with α-chloronaphthalene and α-bromonaphthalene, which they believed comparable to those induced by colchicine. Simonet (1940) also claimed to have produced colchicine-like anomalies with 1,3,5-trinitro-m-xylene when barley and flax seedlings were tested. Shimamura (1939b) used sublimated acenaphthene on young flower buds of *Fritillaria* and observed abnormal nuclear division in pollen mother cells. The pollen mother cells divided into many cells, each containing small nuclei. Hukusima (1939) reported the production of polyploids in *Brassica alboglabra* with the aid of acenaphthene. The flowers were encased in small glass tubes, the walls of which were covered with crystals of acenaphthene. Favorski (1939) listed 12 chemicals among which were aurantia, tribromoaniline, α- and β-naphthylamine, trinitrophenol and tribromophenol. These substances affected mitoses analogous to colchicine. Garrigues (1939) compared the effects of chloral and colchicine on the roots of *Vicia faba*. Chloral, he reported, did not induce swellings of the root; it acted on the resting nucleus, changing its structure, and affected cells above the meristem, thus interfering with the

cells in the elongation zone. Mol (1939) applied acenaphthene in paste form mixed with formalin, alcohol or water to roots of gladiolus. The colchicine-formalin paste killed the roots, while the alcohol mixture injured them. Mol concluded that acenaphthene is less effective than colchicine. Weichsel (1940) used auramin on *Vicia faba* and barley and acenaphthene on *Soya,* luzerne and corn. She reported changes similar to those obtained by Schmuk and Kostoff on the grass seedlings with bromnaphthalene and bromacenaphthalene. Fatalizade (1939), too, showed that crystals of acenaphthene acted on F_1 seedlings of a cross between *N. rustica* × *N. paniculata* and produced polyploids.

COLCHICINE IN GENETICS

Application of colchicine in the study of plant genetics has been productive of new types of plants, the merits of which must still be determined. Blakeslee (1939), who is the leader in this phase of colchicine work, believes that through the application of this alkaloid, new species of plants may be created at will. Blakeslee and Avery (1937) described the various methods of applying colchicine to organs and tissues of the plant. A long series of papers on the effects of colchicine on economic plants has been published. Judicious use of the agent in plant genetic studies has opened a productive field of scientific endeavor and has added a new feature to colchicine little suspected by the early writers. It would be beyond the scope of the present report to review these publications. Attention is directed to a small number of these papers which tend to summarize this vast though new literature and to give some of the general results obtained with colchicine. Emsweller and Ruttle (1941) summarized the use of colchicine in floriculture. Badenhuizen (1941) described the induction of tetraploid economic plants such as tobacco, jute and hemp. Sell (1939) applied colchicine to seedlings of pasture plants such as alfalfa, bur clover, vetches, with satisfactory survival of seedlings. Thompson and Kosar (1939) induced polyploidy in lettuce. Shimamura (1938) produced fertile tetraploid tomatoes. Becker (1938) studied the influence of colchicine on some common vegetables, viz., beans, onions, peas, potatoes, rhubarb and asparagus. Weller (1940) effected chromosome changes in sugar cane seedlings; seeds treated with colchicine showed higher percentage of germination and growth stimulation.

Mendes (1940) described octoploid and tetraploid cotton plants derived from colchicine-treated seeds. Production of tetraploid rubber-producing dandelion (*Taraxacum Kok-saghyz*) was reported by Navashin and Gerasimova (1941). The use of colchicine in fruit breeding was the subject of another report by Nebel and Ruttle (1938b).

COLCHICINE IN CYTOLOGY

Many of the studies on colchicine reported above were accompanied by cytological studies of germ plasm as well as somatic cells, and many cytological investigations *per se* have been made after use of the drug.

Dermen (1940) reviewed the literature on polyploidy as induced by colchicine and has given an adequate summary of the technique employed in the production of these aberrant chromosomal behaviors in plants and animals. Wellensiek (1939) has given a short digest of the use of colchicine in polyploidy as well as an historical account of the properties of the alkaloid. Györffy (1940a, b) studied the chromosome numbers of polyploids obtained by colchicine treatment, and has also summarized the results obtained with colchicine treatment up to 1940. Here only those reports which have a bearing on the problem described will be mentioned.

In normal animal germ cells Nebel and Ruttle saw doubling of chromosomes in fertilized eggs of *Arbacia*. Pincus and Waddington found that rabbit eggs treated with colchicine became tetraploid. Lits (1933) used trypaflavine, arsenicals, mercury, zinc, iodine, bromine and certain toxins to study various tissues of the rat and found that these chemicals induced pycnosis and atypical cell division. An abundance of mitoses was noted in these tissues, 16 hours after injection of 0.025 mg. of colchicine into the rat. These cytological studies were followed by Allen in 1936 who applied colchicine in the study of cellular activity in the endocrines. Nebel (1937) and Nebel and Ruttle (1938) studied the nuclear changes in the stamen hairs of *Tradescantia*. Gavaudan and Gavaudan (1937) treated plants with colchicine and reported arrest of growth with 1/1,000–1/5,000 solution. They observed an increase in chromosome number in the cells studied. In a later paper (1938) they noted that the limit of increase in the number of chromosomes was determined only by the difficulties of nutrition. This they believed could

be surmounted by cultivating plantules or polyploid cells in tissue culture. Gavaudan, P., (1938) studied seedlings of *Vicia faba* which were immersed for two hours, three days in succession in a 1/1,000 solution of colchicine. The plants grew slowly and showed diverse monstrosities of stomata and leaf structures. Tetraploid and even octoploid cells with numerous nucleoli were observed. These changes were associated with interference with the normal karyokinetic process.

Roots of *Allium cepa* were studied by Gavaudan, Gavaudan and Pomriaskinsky-Kobozieff (1937) as affected by colchicine. They found that 0.1 gm. to 0.2 gm. of colchicine per 1,000 cc. in water induced bulbous hypertrophies behind the meristem of the root tips. They reported large numbers of abnormal mitotic figures 40 hours after treatment. Pseudoanaphase stages were noted in which the chromosomes were vaguely arranged in a quadrilateral form (pseudotelophases) with the two chromosomal groups united by a bridge of chromosomal material.

Eigsti (1938–1940), using the smear method, studied the effects of colchicine on the roots of onion, radish and corn seedlings. He found that the cytological changes were determined by the concentration of the colchicine solution, the time of exposure and the activity of the embryonic cells at the time of treatment. Eigsti (1940b) pointed out that chromosome breakage is higher in colchicine-treated material than in untreated cells of the same species. This, he inferred, induced variation other than polyploid changes.

Levan (1938–1939) made studies on the cytological effect of colchicine in the root mitoses of *Allium fistulosum* and *A. cepa*. Levan, too, observed that treated roots ceased to elongate but formed swellings at the root tip meristem, which he described as tumors. The volume of the meristematic cells increased but no new cells were formed. Levan showed that the cells in treated roots transferred to water reverted to normal mitosis. In colchicinized onion root tips he counted 128 and 256 chromosomes. Concentration as low as 0.0055% colchicine caused disturbance of the spindle. The threshold value of colchicine on root mitoses of *A. cepa,* Levan believed, was reached with a 0.005% to 0.01% solution after an exposure of four hours. Nebel (1937) found a 0.004% solution of colchicine effective in plants, while Ludford (1936) showed that a solution of 0.00001% was effective in ani-

mal tissue. Mangenot (1938a, b, 1939) studied roots of *Allium cepa* and *Hyacinthus orientalis* and seeds of *Lupinus albus* grown in Knopf solution to which 1/2,000 to 1/3,000 colchicine was added. He found that the roots remained alive after a long period in the mixture. He observed the tumefaciens effect of colchicine and ascribed it to a defect in the growth of the roots. The number of prophase stages was studied by Mangenot who contended that the number of cells in this stage did not vary up to 102 hours of exposure to colchicine, after which it decreased slowly, while the number of metaphases, anaphases and telophases fell off rapidly to zero. Levine and Gelber (1943) showed that with a colchicine concentration of 0.01% the maximum number of metaphase stages in the root tips of *Allium cepa* was reached after an exposure of 24 hours. Continued exposure to the same solution caused a general reduction in the number of metaphase stages until the number reached in the 140th hour was smaller than that found in normal root tips.

Shimamura (1939a) investigated the effects of colchicine on the roots and seedlings of *Allium cepa, Lycopersicum esculentum* and *L. pimpinellifolium*. He exposed the roots of onion to a 0.4% solution for two hours, after which they were placed in running water for one hour and then fixed at varying intervals. Shimamura described in detail the chromosome behavior in the colchicine-treated onion root tips, and believed that colchicine interfered with the mechanism that pulled the chromatids apart. In the absence of spindle fibers Shimamura described an orientation of the chromosome material about a degenerate spindle substance. The chromosomes became vesicular, they approached each other and formed a dumbbell-shaped body. The two masses of chromosomes connected by a narrow bridge of chromatic material separated at the link and formed two nuclei. In some cases the chromosome masses fused, forming a tetraploid nucleus. The tomato seedlings treated with colchicine produced tetraploid and myxoploid plants. In the roots of these seedlings aneuploidy was seen. Duhamet (1939) attempted to establish the colchicine concentration which would be most capable of producing a reaction in the roots of white lupine grown *in vitro* and attempted to induce a diminution or suppression of these effects. He observed that colchicine in concentration 10^{-4} completely inhibited growth of isolated roots in 10 to 12 days. Ad-

dition of heteroauxine in concentration 10^{-12} to this solution of colchicine interfered with the growth block induced by colchicine and was followed by a return to normal division. Levine and Lein (1941) studied the effect of various growth substances, *e.g.*, vitamine B, indoleacetic acid and colchicine, on root number and length of roots of *Allium cepa* grown in water in which these substances were used singly or in combination. Colchicine 10^{-3} solution inhibited root growth; when followed by exposure to a 10^{-8} solution of indoleacetic acid, stimulation of both formation and linear growth of roots occurred.

COLCHICINE ON THE ENDOCRINES

Allen used colchicine as an aid to determine the cellular activities of various endocrine tissues under normal conditions and under the influence of various hormones. He maintained that the colchicine technique was a new tool for the study of hyperplasias. Allen and Creadick (1937) studied the mitotic activity in the germinal epithelium of mouse ovary during sexual maturity by the use of colchicine. The arrested mitoses delayed the processes so that an adequate account of the activities could be determined. The effects of pituitary extract, prephysin and pregnant mares serum on the germinal epithelium were so interpreted. Allen and Creadick pointed out that the evidence was accentuated by the arrested mitoses after colchicine and that ovogenesis was clearly demonstrated to occur in the ovaries of the mature mouse at oestrus. Allen, Smith and Gardner (1937) studied the effects of theelin on genital tissue of the mouse after ovariectomy. Hyperplasia of the uterus muscle as shown through the retarded mitotic changes induced by colchicine was reported by Allen, Smith and Reynolds (1937). Mitotic figures in the vaginal epithelium of a colchicinized mouse numbered 1,000, while in the untreated animal only 10 to 20 dividing cells could be seen in a single cross section, as shown by Allen (1938). The efficacy of various methods of making vaginal smears could best be shown by the use of colchicine in the rat, as reported by Rogers and Allen (1937). Williams, Stein and Allen (1941) studied the genital tissues of the female mouse after application of colchicine. Colchicine effects were noted in six hours in the uterus and rectum. No effect was observed in spayed mice not treated with oestrone.

Following Allen and his associates, Bastenie and Zylberszac (1937a–d) used colchicine to arrest mitotic activity to better study the effects of the extracts of the hypophysis on the thyroid in young guinea pig, the influence of testosterone propionate on the genital apparatus of the rat and the effect of extracts of the antepituitary on the reproductive organs of the immature female guinea pig. The stimulative effects of antehypophysis on the parathyroids in the rat were demonstrated by the delaying action of colchicine by Bastenie and Zylberszac (1938). Leblond and Allen (1937) studied the effects of prolactin on the crop glands of pigeon by the use of colchicine. In a later paper, Leblond and Segal (1938) reported the effects of colchicine on adrenalectomized rats. The adrenal, he contended, suppressed the effects of colchicine. The lethal dose was one-third as strong for the adrenalectomized animal as for the control. Freud and Uyldert (1938) compared the intestines of normal and adrenalectomized rats after injection with cortin. The delayed nuclear division after colchicine injections showed that adrenalectomy had no effect on mitoses in the epithelium. Adrenalectomy did not interfere with mitotic activity in the intestine. Jailer (1938) applied colchicine in an effort to determine the mitotic index in the interstitial cells of guinea pig under the influence of the gonadotropic hormone. Bureau (1939) found, with the aid of colchicine, that oestrone and progesterone augment each other in their effect on the epithelium of the uterine horns of rabbit, as shown by mitotic increase. Androgenic treatment applied to castrated rats was studied by Burkhardt (1939) to determine its effects on the ventral prostate. Testosterone propionate was injected in 80-day-old castrated animals. The mitotic activity in the ventral prostate appeared at its best six hours after the injection of colchicine. Schmidt (1942) studied the ovary of the normal mature guinea pig after treatment with colchicine. Increase in mitoses was observed in the granulosa and theca interna and theca externa. The follicules were also increased in size. DuBilier and Warren (1941) studied the mitotic activity in the Brown-Pearce rabbit epithelioma with application of single and repeated small doses of colchicine. The number of mitotic figures for a given dose and given time after injection varied so that in general the epithelioma showed no consistency in its response to colchicine.

IRRADIATION WITH X-RAYS

It has been generally conceded that x-rays and the B and Y rays of radium are valuable adjuncts to surgery if not self-sufficient agents in the effective treatment of neoplastic diseases of man. Many roentgenologists believe that the efficacy of these rays lies in their destructive action on dividing cells. The resting stages have been considered resistant. However, there are two principal groups of investigators whose studies deal primarily with these highly important problems. Strangeways and his associates, Oakly and Hopwood, observed that irradiated tissue cultures showed a decrease in the number of dividing cells. They observed that this decrease was due temporarily to inhibition of the onset of mitoses. These views were supported by Kemp and Juel. On the other hand, Mottram, Richards, Grasnick, Holthusen, Alberti and Politzer and many others contended that cells are most sensitive to irradiation while in the process of mitosis. Love's (1931) studies on choroid and sclerotic tissues of embryo chicks of eight to nine days attempted to analyze the effects of x-rays on dividing cells. He studied the percentage of survival of dividing cells immediately after irradiation, and concluded that reduction in the number of cells in mitosis after irradiation was due to inhibition of some factor of those cells which normally would have entered mitosis during irradiation. He stated that the radio-sensitivity of a cell was a function of its displacement from maturity. When the displacement of a cell from its maturity was less than 100 minutes, the radio-sensitivity was constant and independent of displacement. When the displacement of a cell from its maturity was greater than three hours, its radio-sensitivity decreased. Studies on the effects of chromosomes of animals and plants have been numerous. Here, only the recent literature dealing with the application of irradiation to onion root tips will be stressed.

Nichols (1941), working with Sax, investigated the chromosomes of untreated root tips of onion seedlings. He reported that chromosome aberrations were frequent in very young seedlings, while in older ones few chromosome abnormalities were found. Nichols believed that cells with chromosome aberrations were eliminated by differential survival of the normal and abnormal cells. Marshak and Hudson (1937) used onion seedling root tips as a biological method of measuring roentgen ray dosage. They be-

lieved that the biological response per roentgen (r) is independent of wave length over the region studied. They used small doses, 40r to 346r. Marquardt (1938) studied the effects of soft rays on *Scilla campanulata* root tips and pollen mitosis in *Bellevalia romana.* He described two kinds of effects which he called primary and secondary. The first effect occurred in such nuclei as, at the time of irradiation, were found in mitosis or about to enter that state. The primary effects were described as clumping and fragmentation of chromosomes, lack of spindle formation and indefiniteness of division polarity. The period following the primary effects was a mitosis-free interim after which the secondary effects became apparent. In the pollen studies there was no mitosis-free interim. The secondary effects brought about chromosome injuries, fragmentations and restitution processes, lateral chromatid translocations, ring-forming injuries and inversion forms. Villars (1940) studied the effects of x-irradiation on colchicine-treated roots of *Pisum sativum* and *Allium cepa* seedlings. They were placed in a solution of 1:2,000 colchicine and irradiated after 40, 48, 96 and 120 hours of immersion. Their treatment had no effects on stages up to and including prophase. In the following stage the chromosomes agglutinated and fused into one mass, and spindle formation was inhibited.

Sax (1941) studied the effects of x-irradiation on the roots of *Allium cepa.* While the primary purpose was to describe the chromosome changes induced by the x-rays, an exposure to 0.1% colchicine for 24 hours was applied to facilitate this study. Aberration frequency was based on the number of chromosomal abnormalities per hundred cells counted. Sax described primary and secondary effects produced by x-rays which were in accord with Marquardt's observations. The clumping and fragmentation of chromosomes after the treatments given by Marquardt support the contention of Dustin and his workers that colchicine had a radiomimetic effect. Sax's experiments did not differentiate between the effects induced under colchicine and those by the x-rays. Sax showed that high x-ray doses suppressed nuclear division from one to two days, while low x-ray doses retarded nuclear activity and the chromosome clumping persisted for several days. The primary effects were evident, Sax claimed, on the metaphase stage. The secondary effect of x-rays involved the production of typical chromosomal aberra-

tions which consisted of chromosome breaks, usually followed by chromosome union to produce rings, dicentrics, inversion, translocations and interstitial deletions. The secondary x-ray effects, Sax believed, persisted for several cell generations, but were soon eliminated, and cells with normal or balanced genoms survived. Sax used doses of x-rays from 150r to 600r. Larger doses of 1,500r to 5,000r were tried on seedlings of ornamental plants; the highest dosage killed or retarded the seedlings of most species studied.

SUMMARY

It has been shown that colchicine administered or applied in small doses to plants or animals produces a retarding effect on the mitotic process of cells in either growing vegetative or germinal tissue. The effect is not one of growth stimulation but one of growth delay with resulting hypertrophy of the affected cells. The nuclei in these cells fail to complete the division process, for they become arrested after the chromosomes divide (metaphase stage). In colchicine-treated mammalian tissue the metaphases begin to accumulate in two to three hours and reach the maximum number in ten to fifteen hours. In cold blood animals, the metaphase stages gather much more slowly and the maximum number occurs after five to seven days. In roots of *Allium cepa* the metaphases reach a maximum after an exposure to 0.01% solution in about 24 hours.

Hypertrophy of the cells apparently begins soon after the metaphase stage is reached. After the 24-hour exposure the cells with their doubled number of chromosomes begin reconstruction of their nuclei and atypical ones are formed. This phenomenon becomes very prominent after 48-hours exposure to this drug. At this concentration of the alkaloid, death is not produced, for the roots recover when they are washed thoroughly and immersed in fresh tap water. The hypertrophies, however, do not disappear and growth of the meristematic tissue is resumed.

The area of a plant inoculated with the *B. tumefaciens*, when covered with a lanolin paste to which colchicine is added, does not prevent this plant tumor from developing. When this paste is applied to a well formed gall, death of the overgrowth results.

Malignant tumors of animals are characterized by numerous cells in nuclear division. Injection of colchicine, in appropriate solution, into the cancerous animal emphasizes the division phase in the

cells of the tumor. The many chromosome aberrations found in malignant cells (Levine, 1931) are analogous to those induced by colchicine. The young diploid peripheral cells become arrested in metaphase, and hyperchromatic cells typical of the older parts of the cancer are produced.

The now well established facts that colchicine arrests nuclear division in a stage where the chromosomes are prominent features of the cells, and that these chromosomes remain in the cells as distinct bodies for longer periods of time and that many more cells attain this stage than can be found in untreated tissue, make the use of colchicine of great importance in the treatment of neoplastic diseases. It is accepted by some roentgenologists that the cell in division is more vulnerable to x-rays or radium than when in resting stage. Continued application of the poison in the treatment of this disease raises the colchicine level to the lethal point and causes death. Repeated application of x-rays and radium to deep seated growths has not been conclusively successful. The suggested rationale involves the use of both agents. Continued use of colchicine and x-rays has been successful in some animal work. In cancer of man insufficient tests have been made. The cases used were in terminal stages.

The report on the effect of combined colchicine and x-ray treatments to fundamental tissue like the root tip of onion, will appear elsewhere. The study on simple tissues should serve as guide for a more intensive study on animal tumors. Crown galls on economic plants may be treated more effectively by preventive methods which will avoid the introduction of the tumor-producing organism.

In endocrinology, colchicine has proved to be an aid in the study of normal growth processes, while in plant breeding the influence of colchicine on seeds and buds has been productive of some useful economic plants. Genetically, according to Blakeslee, colchicine has given the geneticist a tool for the production, at will, of new species of plants.

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